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ANTIMICROBIAL EFFECT OF ORANGE JUICE, PEEL AND IT'S ESSENTIAL OIL ON THE SHELF LIFE OF CAKE

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ABSTRACT

The objective of this work was to study the effect of orange juice, peel and it's essential oil as antimicrobial agent, on the shelf life of cake when stored at room temperature and at 4°C for 4 weeks. Sensory evaluation of different cup cake types produced from wheat flour containing different levels of orange peel and essential oil showed that there are non significant differences in taste and flavour between treatments (control with orange), orange peels 2.5% + orange juice, orange essential oil 0.3% + orange juice and control. The cake made with mixture of 0.3% orange essential oil + orange juice gave products of low pH (7.16), but cake with a mixture of orange juice and orange peel 2.5% plus milk gave the lowest a_w (0.88).

All pathogens were not detected in all samples stored at 4 °C, while small numbers of *Staph aureus* and *B. cereus* were detected upon 21 days of storage at 30 °C. The inhibition zones against strains of Asp. *Niger sp. flavus, sp parasitius, sp ocfiracus*, Fuarium moniliforme and *pencillum spp*, showed that orange oil had the highest inhibitory effect all tested followed by the effect of orange peels, then orange juice. Generally it could be clearly all additives had considerable effects on the growth inhibition of all fungal tested strains, while, essential orange oil had the most inhibitory effect causing undetected cells in some strains from *As. sp.* and *Penoillium spp.*

INTRODUCTION

In bakery processing, the most common type of microbial spoilage is mould growth which governs shelf life (Earle and Putt, 1984). As products of intermediate moisture (0.75 – 0.90aw being the water activity) (Marin *et al.*, 2002), cakes are particularly sensitive to xerophilic moulds spoilage (Fustier *et al.*, 1998).

Citrus oils not only devotes themselves to be used in food but also are generally recognized as safe (GRAS) and have been found to be inhibitory both by indirect oil and vapour form against a range of both Gram-positive and Gram-negative bacteria. Citrus oils may provide the natural antimicrobials that the food industry requires to fulfill both its requirements and those of the consumer (Fisher and Phillips, 2008).

Flavoring materials are present in both orange juice and peel. Numerous studies were carried out concerning the volatile composition of orange juice as well as several recent GC-O studies of orange juice aroma (Bazemore *et al.*, 1999).

The bakery products and flour confectionery sector has witnessed intense technological progress which has brought many changes in process innovation, and in commercial and qualitative characteristics of the products. Usually, bakery products are packaged in plastic films after baking and cooling and they are consumed within 1 or 2 month (Ponte and Tsen, 1987).

Some volatile components of citrus fruit essential oils show a high degree of fungal inhibition (Caccioni *et al.*, 1998).

Researchers are looking for new sources of ingredients and/or additives. One such source may be agro-food by-products. In the production of these new ingredients other, new by-products are generated, such as when fiber-rich citrus extracts are obtained (Lario *et al.*, 2004). These extracts have been shown to have antimicrobial properties in several foodstuffs (Fernandez-Lopez *et al.*, 2005). Citrus essential oils (EOs) contain 85-99% volatile and 1-15% non-volatile components. The volatile constituents are a mixture of monoterpene (Limonen) and sesquiterpene hydrocarbons and their oxygenated derivatives including: aldhydes (citral), ketones, acids, alcohols (linalool) and esters (Flamini *et al.*, 2007).

Accordingly,This study was a trial to evaluate the efficiency of orange juice, peel and essential oil as antimicrobial agents in cake samples during storage period at room temp. and 4 °C for 4 weeks.

MATERIALS AND METHODS

a - Materials

- Orange essential oil was obtained from Harris Company. Wheat flour 72% extraction rate and other ingredients were purchased from the local market at Giza, Egypt.

- Eight strains of pathogenic fungi belonging to the American Type Culture Collection (ATCC) were obtained from culture collection of the Microbiology Dep., Fac. Agric; Cairo. Univ. Egypt. These strains c ultures were maintained on malt agar slants at 4 °C and sub-culture at 25 °C in nutrient broth for 24 hrs prior to inoculation.

b - Methods

Media Preparation

The basic medium used was a 2% wheat flour agar. It was adjusted to achieve the desired aw level and pH 6 (mean pH for bakery products), as previously described by (Marin *et al.*, 2002). Sterile media were pored into 9-cm diameter sterile Petri dishes. The final a_w and pH levels of the media were checked.

C – Cake preparation

Cup cakes were prepared according to the standard formula method (A.A.C.C., 1983), by substituting milk (as control with the investigated orange components), as follow :- orange essential juice (1), orange peel 2.5% + milk (2), orange peel 2.5% + orange juice (3), orange essential oil 0.3% + milk (4) and orange oil 0.3% + orange juice (5).

d – Sensory evaluation

Cake samples were sensory evaluated by 10 panelists for various quality attributes such as texture, moistness, tenderness, softness, crumb color and flavor according to the methods described by (A.A.C.C. 1983). pH values were determined during storage periods (7, 14, 21 and 28 days), according to the method described by (Tellez *et al.*, 1988).

e- Microbial examination

Total aerobic bacterial counts, aerobic spore forming bacteria, mold-yeast counts, *Salmonella, Staph aureus, E. Coli, Bacillus cereus* were determined according to (Al-Mohizea *et al.*, 1987). All samples of cake were examined daily for visible mold growth (spoilage), and the time elapsed before appearance of mold which was considered to be MFSL.

RESULTS AND DISCUSSION

Using of orange juice, peel and essential oil as antimicrobial agents in food might affect organoleptic properties of the cake, so that finding an essential oil, peel and juice that has greatest effect at the lowest concentration is essential.

Table (1) : Determination of fungal inhibition zones (mm) as affected by orange juice, Peel and essential oil .

Type of pathogens	Zone inhibition (mm)			
	Juice	Oil	Peel	Peel & Juice
Aspergillus oryzea	3	15	8	10
Aspergillus niger	4	15	9	12
Aspergillus flavus	2	14	7	9
Aspergillus parasiticus	2	12	6	9
Fusarium moniliforme	1	13	6	7
Alternaria spp.	1	13	6	8
Aspergillus ochracus	3	12	6	8
Pencillium spp.	6	18	8	13

Table (1) shows the inhibition zones as affected by some natural additives, e.g. orange juice, peel and its essential oil or orange juice with milk against strains of *Asp. niger, Asp. oryzea* using diffusion technique were measured in mm after 24 hours of incubation at 25 ± 1°C.

Data in Table (1) reveal that all types of additives used in this study had antifungal potential with the orange essential oil having the highest effect in this respect, specially its inhibitory effect on *Penicillium spp.* As mentioned above, orange essential oil had the highest inhibitory effect followed by orange peel and juice.

Data in Table (2) point out that the treatments D and E gave the best results, since there was no detectable growth of *Asp. oryzea, Asp. niger, Asp. flavus or Pencillium spp.* Treatments H and G gave the following best results of *Pencillium spp.* compared to the ones mentioned above. Both D and E treatments which contain the highest levels of orange essential oil, 200 and 300 microliter, respectively, had the lowest counts from most strains and disappeared cells (about 50% from all strains). In case of orange juice and peel, data explain that, increasing levels of orange juice and peel (F, G and H treatments) decrease cell counts for all fungal strains, compared with control (Treatment A).

Table (2) :Effect of orange juice and essential oil on total fungal viable count at 25 ± 1°C .

Treatme	Viable counts (cfu/g)							
	Asp. oryzea	*Asp. niger*	*Asp. flavus*	*Asp. parasiticus*	*Fus. moriliforme*	*Alter. spp.*	*Asp. ochracus*	*Penicillium spp.*
A	$40x10^6$	$54x10^6$	$28x10^6$	$50x10^6$	$56x10^6$	$55x10^6$	$60x10^6$	$55x10^6$
B	$43x10^4$	$49x10^5$	$48x10^4$	$42x10^4$	$60x10^4$	$54x10^4$	$45x10^5$	$32xx10^4$
C	$30x10^2$	$43x10^3$	$39x10^3$	$48x10^3$	$28x10^3$	$30x10^3$	$30x10^3$	$18x10^3$
D	ND	ND	ND	15x10	8x10	10x10	8x10	ND
E	ND	ND	ND	12x10	6x10	7x10	9x10	ND
F	$38x10^3$	$44x10^4$	$41x10^4$	$56x10^4$	$30x10^4$	$23x10^4$	$49x10^4$	$23x10^3$
G	$17x10^2$	$18x10^2$	$25x10^2$	$44x10^2$	$30x10^2$	$37x10^2$	$40x10^2$	$12x10^2$
H	$12x10^2$	$13x10^2$	$20x10^2$	$38x10^2$	$36x10^2$	$35x10^2$	$33x10^2$	$10x10^2$

A : control which is consisted of a liter of malt agar +440gm glecerol + 20 gm flour, B: 50 microliter orange oil per liter media, C : 100 microliter orange oil per liter media, D:200 microliter orange oil per liter media, E: 300 microliter orange oil per liter media, F: 1% orange juice + 1% peel per liter media,G:2.5 % orange juice + 2.5 peel per liter media, H: 5 % orange juice +5% peel per liter media,

From the above mentioned data, it could be concluded that all additives had a considerable effect on the growth inhibition of all fungal strains. While essential orange oil had the highest inhibitory effect causing undetected cells for some strains of *Aspergillus sp.* and *Pencillium sp.*

The possible use of citrus oil against growth of *Aspergillus flavus* was assessed at pH values of 3.5 and 4.5 at 25 °C in water of 0.99 or 0,95 (aw). pH had no effect on the Minimum inhibitory concentrations (MICs), but water activity was effective, with 1800 ppm needed for aw of 0.99 and 1400 – 1500 ppm aw of 0.95 (Lopez-Malo *et al.*, 2005).

Citrus peel oils also reduce the levels of *Aspergillus parasiticus* at levels of 1.6% over a 10 days incubation period (Sharma & Tripathi, 2006). The essential oils of lemon, orange, mandarin and grapefruit at levels of 0.27%, 0.47%, 0.71% and 0.94%, respectively, inhibited the growth of the mould *Asp.niger* and *Asp.flavus*, *Penicillium chry sogenum* and *penicillium verrucosum* (Viuda-Martos *et al.*, 2008). Also, they found that orange essential oil the greatest reduction in mycelium growth of this fungus (29.5%, 36.4% and 48.1%) at levels of 0.27%, 0.47% and 0.71% percentage reduction of respectively

The results given in Table (3) show that non significant difference in taste and flavor between treatments (1), (3), (5) and control were detected. Also, it has been noticed that no significant differences in other properties between control and other treatments. It could be concluded from given results that using of citrus oils as antimicrobials in food might affect organoleptic properties of the food stuff, so that oil component vapour that has the greatest effect at lowest levels is essential. Odour cross matching tests have been carried out on citrus oils to establish the contributory flavour compounds that relate to food aroma (Chida *et al.*, 2006).

Table (3): Effect of orange juice, peel and essential oil on sensory properties of cup cake.

Treatment	Tast Cake / Formula Using	Taste & Flavor 10	Crumb color 10	Grain 10	Moistness 10	Texture			Cells	
						Tenderness 14	Softness 10	Uniformity 10	Size of cells 10	Thickness of walls 10
	Control with milk	15.30 ^{A}	9.55 ^{A}	9.55 ^{A}	9.25 ^{A}	12.25 ^{A}	9.15 ^{A}	9.20 ^{A}	9.00 ^{A}	9.00 ^{A}
1	Control with orange juice	14.80 ^{BA}	8.75 ^{B}	8.90 ^{BA}	8.95 ^{A}	11.95 ^{A}	8.65 ^{A}	8.15 ^{B}	8.35 ^{A}	8.60 ^{A}
2	Orange peel 2.5% + milk	14.00 ^{BA}	8.85 ^{B}	8.90 ^{BA}	8.85 ^{A}	12.20 ^{A}	8.60 ^{A}	8.60 ^{BA}	8.40 ^{A}	8.90 ^{A}
3	Orange peel 2.5% + orange	14.40 ^{BA}	8.75 ^{B}	8.70 ^{BA}	9.25 ^{A}	12.55 ^{A}	9.00 ^{A}	8.65 ^{BA}	8.30 ^{A}	9.20 ^{A}
4	Milk + orange oil 0.3%	14.10 ^{B}	8.65 ^{B}	8.70 ^{B}	8.70 ^{A}	12.00 ^{A}	8.50 ^{A}	8.60 ^{BA}	8.30 ^{A}	8.90 ^{A}
5	Orange oil 0.3% + orange juice	14.30 ^{BA}	9.25 ^{BA}	8.9 ^{BA}	9.05 ^{A}	12.45 ^{A}	8.70 ^{A}	8.70 ^{BA}	8.65 ^{A}	8.90 ^{A}
	LSD	1.0644	0.681	0.943	0.694	1.4864	0.8718	0.897	1.015	0.8248

Tzortzakis (2007) reported that the use of citrus oil in food may be a way of combating some of the negative organoleptic implications associated with EOs, as there would be no direct contact between the concentrated oil and the food stuff.

Table (4): The water activity and pH values of cake at zero time

Additives to Cake formula	a_W (water activity)	pH values
Control with milk	0.91	7.88
1- Control with orange juice	0.88	7.26
2- Orange peel 2.5% + milk	0.88	7.77
3- Orange peel 2.5% + orange	0.90	7.62
4- Orange oil 0.3% + milk	0.89	8.59
5- Orange oil 0.3% + orange juice	0.90	7.16

Data in Table (4) indicate that the cake made with 0.3% orange oil plus orange juice gave the least pH value of the product, indicating the longest shelf-life of this product, it must be taken.

From the same table, it can be also seen that the treatments which contained 2.5% orange peel + milk and 2.5% orange peel+ orange juice gave the lowest a_W (0.88 and 0.88 a_w, respectively). However, a wide a_W range has been used in this study (0.80 – 0.95). Most common bakery products have a_W values in the range of (0.70 – 0.85) which restricts fungal growth. Under low a_W condition, the situation is more optimistic as total control of growth may be achieved at low pH (Marin *et al.*, 2002).

Table (5) : The total different microbial counts in cake types (milk & orange) during storage at both 30°C and 4°C.

Treat-ment	Storage time (days)	Total bacterial counts		Spore formers		Yeasts & Molds		*Staphyloc occus aureus*		*Bacillus cereus*		*Esherachia coli*	
		30°C	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C	4°C
A	٧	$10x10^2$	1x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A	١٤	$68x10^3$	3 x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A	٢١	$64x10^5$	38x10	$34x10^2$	ND	$43x10^2$	ND	28x10	ND	32x10	ND	ND	ND
A	٢٨	sp	$28x10^2$	sp	30x10	sp	30x10	sp	ND	sp	ND	sp	ND
A_1	٧	32x10	1.5x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A_1	١٤	34x10	2x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A_1	٢١	$59x10^4$	13x10	35x10	ND	35x10	ND	24x10	ND	26x10	ND	ND	ND
A_1	٢٨	sp	23x10	sp	6x10	sp	6x10	sp	ND	sp	ND	sp	ND
A_2	٧	6x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A_2	١٤	20x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A_2	٢١	39x10	4x10	5x10	ND	5x10	ND	ND	ND	ND	ND	ND	ND
A_2	٢٨	sp	5x10	sp	ND	sp	ND	sp	ND	sp	ND	sp	ND

A: control cake natural recipe + milk , A_1: treatment cake natural recipe + milk +2.5% orange peel , A_2: treatment cake natural recipe + milk +1.0 % orange peel , ND: not detected microbial counts,sp : spoilage sample

Table (5) shows the total bacterial counts of yeast and mold, spore forming and some pathogens (*B.cereus, Staph. areus and E.Coli*), were present which in processed cake, with milk (control), milk and 2.5% orange oil

(A1), and milk 1% orange peel (A2) which were stored at refrigerator and room temperatures.

The highest numbers of total bacterial counts were observed in control sample then treatment (A1), while treatment (A2) had the lowest numbers during the storage period at 30 °C. All samples were spoiled after 28 days. At 4 °C the viable plate counts were not detected in treatment (A2) which were remained constant in their small numbers in control and treatment (A1) (102 and 101respectively). In case of spore forming and yeast and mold ,they had similar results for both temperatures.

For samples kept at 30 °C, spore forming bacteria were not detected until 21 days of storage. The score of control was 102 cfu/gm, where it reached 350 cfu/gm I1 but it was only 50 cfu/gm for A2. Scoring the bacterial counts after 28 days of storage resulted in 102 cfu/gm for control, 350 cfu/gm for treatment A1 and only 50 cfu/gm for treatment (A2). Under refrigeration and storage for 28 days, no spore forming bacterial counts were detected in treatment (A2), while 60 cfu/gm were detected for treatment (A1) and 300 cfu/gm for control.

All pathogens were not detected in all samples stored at 4 oC, while small numbers of both Staph aureus and B. cereus were detected upon storage for 21 days at 30 oC.

It was found that the phenolic compounds showed a lesser antimicrobial activity, thought to be due to their poor solubility in water. The water could also reduce their volatility as compounds with hydroxyl groups may be more solvated and remain in water phase (Stato *et al.*, 2006).

Table (6) : The total different microbial counts in cake types (milk & orange) during storage at both 30◦C and 4◦C.

Treatment	Storage time (days)	Viable counts (cfu/g) at:											
		Total bacterial counts		Spore formers		Yeasts & Molds		*Staphyloc oc-us aureus*		*Bacillus cereus*		*Esherachia coli*	
		30◦C	4◦C	30◦C	4◦C	30◦C	4◦C	30◦C	4◦C	30◦C	4◦C	30◦C	4◦C
B	٧	20x10	0.5x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B	١٤	$12x10^2$	2x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B	٢١	$30x10^5$	21x10	21x10	1x10	21x10	ND	12x10	ND	15x10	ND	ND	ND
B	٢٨	sp	33x10	sp	18x10	sp	30x10	sp	ND	sp	ND	sp	ND
B_1	٧	12x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B_1	١٤	34x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B_1	٢١	70x10	5x10	8x10	ND	8x10	ND	ND	ND	ND	ND	ND	ND
B_1	٢٨	$55x10^2$	7x10	10x10	ND	15x10	6x10	ND	ND	ND	ND	ND	ND
B_2	٧	4x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B_2	١٤	11x10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B_2	٢١	22x10	2x10	2x10	ND	5x10	ND	ND	ND	ND	ND	ND	ND
B_2	٢٨	$44x10^2$	7x10	5x10	ND	7x10	ND	ND	ND	ND	ND	ND	ND

B control cake natural recipe + orange, B_1: treatment cake natural recipe + orange+2.5% orange peels

, B2 : treatment cake natural recipe + orange+1.0 % orange peels , ND: not detected microbial counts,

Results in Table (6) point out that treatment (B2) which cake made with natural recipe plus orange to which 0.3% orange peels were added was superior in total different microbial counts to the control and treatment (B1). However, in general, the results from this experiment were not as expected. Cake dough lipids may affect the activity of essential oils because of the hydrophobic properties of their active compounds (Arras and Usai, 2001). Some studies suggested that compounds penetrate inside the cell, where they interfere with cellular metabolism (Marino *et al.*, 2001). Other studies suggested that they disturb the cellular membrane and react with active sites of enzymes or act as a H^+ carrier, depleting Adenosine Triphosphate pool (Ultee *et al.*, 2002). As a conclusion, volatile component of orange essential oil had a potential antifungal activity against common fungi causing spoilage of bakery products, due to the poor activity observed when they were used in a sponge cake. Further studies are needed before confirming their application in cake.

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تأثير عصير البرتقال والقشر و الزيت كمضادات ميكروبية على فترة الحفظ للكيك

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يهدف هذا البحث الى دراسة تاثير عصير البرتقال والقشور وزيت قشور البرتقال كمضادات ميكروبية على درجة حرارة الغرفة وكذا على درجة حرارة ٤ °م والمخزنة لمدة ٤ أسابيع. أظهرت نتائج التقيم الحسى لآنواع الكيك المختلفة المنتجة من دقيق القمح المحتوى على مستويات مختلفة من العصير وقشر البرتقال وزيت قشر البرتقال انة لايوجد أختلافات معنوية على مستوى ($p > 0.05$) فى الطعم أو الرائحة بين المعاملة (المعاملة القياسية مع البرتقال) ، قشر البرتقال ٢,٥% + عصير البرتقال، زيت قشر البرتقال ٠,٣% + عصير البرتقال مع مقارنة بالكنترول(المعاملة القياسية مع اللبن) الكيك المصنع من ٠,٣% زيت قشر البرتقال بالاضافة الى عصير البرتقال أقل درجة pH (٧,١٦) من المنتجات بينما أعطى الكيك المصنع من مخلوط عصير البرتقال وقشرالبرتقال ٢,٥% بالاضافة الى اللبن أقل معامل النشاط المائى (٠,٨٨). لم يتم أكتشاف أى كائنات مرضية فى كل العينات المخزنة على على درجة حرارة ٤ °م ، بينما تم أكتشاف على أعداد قليلة من ***Staph aureus*** و الـــ ***B.cereus*** عند التخزين لمدة ٢١ يوم على درجة حرارة ٣٠ °م.

كذلك أظهرت النتائج وجود تثبيط ضد سلالات من ***parasitius,Asp ocfiracus,*** ***Fusarium moriliforme*** and ***Pencillum spp*** وأن زيت قشر البرتقال له أعلى تأثير متبط يلية تأثير قشر البرتقال ثم عصير البرتقال.

وقد أظهرت النتائج أن كل الآضافات لها تأثير كبير على تثبيط نمو سلالات الفطر التى تم دراستها، بينما كان التأثير التثبيطى للزيوت الآساسية لزيت قشر البرتقال لها أعلى تأثير و المسبب لعدم أكتشاف الخلايا لبعض السلالات من ***As.sp.*** والـــ ***Pencillum sp.*** .